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Voltage programming in capillary zone electrophoresis

Huan-Tsung Chang and Edward S. Yeung

Ames Laboratory-US Department of Energy and Department of Chemistry, Iowa State University, Ames, IA 5001 I (USA)

ABSTRACT

Temperature is an important factor in capillary zone electrophoresis since it affects the viscosity and the pH of the buffer solution. In this study, a capillary tube with a large radius (130 μm I.D.) and filled with buffer at a high ionic strength is used to generate substantial joule heat within the capillary tube to force a significant increase in temperature, in turn to decrease the viscosity and to change the pH of the buffer solution. From a study of the degree of dissociation of analytes at different voltages, we show that voltage-induced pH change is significant in 0.1 M tris(hydroxyamino)methane (THAM) but not in 0.025 M hydrogencarbonate buffer system. A step change in voltage from 15 to 25 kV is implemented to generate a pH gradient in the THAM buffer solution. The results show that the method is useful for separating phenols which cannot be separated at a fixed voltage.

INTRODUCTION

Temperature [1,2] is an important parameter in capillary zone electrophoresis [CZE] since it affects not only the flow through convection, but also ionization of the analyte and the capillary surface, the viscosity and the pH of the buffer solution. The effects are smaller than 0.5% per degree for all these terms except for viscosity which has a 2% per degree change. In CZE, temperature control is often used to provide efficient heat removal [3,4]. Manipulation of chemical equilibria such as metal chelation and micelle partitioning [5,6] within the capillary tube through temperature control has also been proposed.

pH is also an important factor to determine selectivity in CZE since it will affect the dissociation of analytes and ionization of the capillary surface, which in turn changes the electrophoretic mobility of charged analyte and the electroosmotic flow coefficient [7,8]. pH gradients have been used to improve the separation process. Boček and co-workers [9,10] used a two-buffer system to force the mi-

gration of varying ratios of two ions into the capillary during separation. Although pH change as a function of temperature is insignificant for most buffers, it is possible to generate a substantial pH change if a buffer system with a large temperature (T) coefficient (dpH/dT) is used. Whang and Yeung [11] demonstrated the effect of temperature-induced pH change on the separation of dyes. In their study, tris (hydroxyamino)methane (THAM) buffer which has a large temperature coefficient is used to generate a pH gradient via controlling the temperature of the column.

Joule heat is evolved when electrical current passes through the capillary tube. This is usually not desirable because it will distort the zone distribution. However, it is possible to increase the temperature significantly by using a high concentration of buffer, running at a high voltage and using a capillary tube with a large radius [12]. One can then dramatically decrease the viscosity and change the pH of the buffer. As viscosity decreases, the electrophoretic mobility and electroosmotic flow coefficient will increase. Thus, shorter separation time and better resolution may be achieved. Altering the pH further modifies the selectivity for components such as weak acids and bases.

In this paper, the effect of Joule heat on CZE is

Correspondence to: Dr. E. S. Yeung, Ames Laboratory-US Department of Energy and Department of Chemistry, Iowa State University, Ames, IA 50011, USA.

examined. THAM (with large dpH/dT) and hydrogencarbonate (with small dpH/dT) are used to study the effect of voltage-induced pH change on the separation of phenols. We also show the implementation of a step change in voltage from 15 to 25 kV to generate a pH gradient for separating phenols in 0.1 A4 THAM buffer system.

THEORY

Separation in CZE is dependent on the electroosmotic flow coefficient and the electrophoretic mobility. Careful consideration of the factors that affect these parameters is important to obtaining good separations. Eqn. 1 [13] can be used to relate the decrease in electromomostic flow coefficient, m_{eo}, to the increase in viscosity

$$m_{eo} = \varepsilon \zeta_c / 4\pi\eta \tag{1}$$

where ε is the dielectric constant, ζ_c is the ζ potential at the slipping plane and η is the bulk viscosity. Electrophoretic mobility, m_{ep}, can be given by [14]

$$m_{ep} = \varepsilon \zeta_a / 6\pi\eta \tag{2}$$

where ζ_a is the ζ potential of the analyte. Eqns. 1 and 2 show that both the electroosmotic flow coefficient and the electrophoretic mobility increase when the viscosity of the buffer decreases. In order to force fast flow, low viscosity of the buffer is needed. Temperature is an important factor in determining the viscosity of the buffer solution. They are related by

$$1/\eta = A \, e^{-B/T} \tag{3}$$

where A and B are constants related to the medium. From eqn. 3, fast flow will be achieved at high temperatures.

Since the effective electrophoretic mobility, m_{eff}, is proportional to the fraction of free ion of the analyte, Tiselius (see ref. 15) derived the equation

$$m_{eff} = \sum \alpha_i \, m_{ep} \tag{4}$$

where α_i is the degree of dissociation and m_{ep} is the absolute mobility of the ith ionic form of a molecule. To relate the effective electrophoretic mobility, degree of dissociation for a monovalent ion and viscosity of the bulk solution, eqn. 4 can be combined with eqn. 2 to give

$$m_{eff} = \alpha_i \varepsilon \zeta_a / 4\pi\eta \tag{5}$$

In order to simplify the problem for estimating the changing mobilities of an analyte ζ_a is assumed to be constant at different voltages. The ratio of mobilities at different conditions i and j is then

$$(m_{effi}/m_{effj}) = (\alpha_i/\alpha_j)(\eta_j/\eta_i) \tag{6}$$

In this study, η_i/η_j can be calculated from the m_{ep} of benzoic acid at different voltages since it is completely dissociated over the range of pH used.

Since the temperature of the capillary tube is an important factor in establishing the viscosity, pH of the buffer, and the degree of dissociation of the analyte, it is important to know the temperature of capillary tube. It can be estimated by eqn. 7 [16]

$$T = 1820/[\ln(m_{eo1}) - \ln(m_{eo2}) + 6.1 \; 1] \tag{7}$$

where m_{eo1} and m_{eo2} are the electroosmotic flow coefficient at 298 K and T K. Therefore, T can be determined by measuring m_{eo1} at a low voltage where we assume little heat is generated, and then measuring m_{eo2} at a high voltage. As soon as the temperature of capillary tube is determined, the pH of the buffer can be calculated from the temperature coefficient of the buffer. Then, α can be estimated from eqn. 8

$$\alpha = K_a/(K_a + [\text{H}^+]) \tag{8}$$

where K_a is the dissociation constant of the analyte.

EXPERIMENTAL

A commercial electrophoresis instrument (lsco Model 3850, Lincoln, NE, USA) was used for all electrophoretic experiments. The fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was 55 cm × 130 μm I.D. At 35 cm from the injection end. the polyimide coating was burned off to form the detection window. A digitizer (Data Translation Model DT 2802, Palo Alto, CA, USA) and a computer (PC/AT, IBM, Boca Raton, FL, USA) were used to collect and store all of the data.

All chemicals were of reagent grade and were obtained from Aldrich (Milwaukee, WI, USA), except that THAM and sodium hydrogencarbonate were from Fisher (Fair Lawn, NJ, USA). Buffer solutions were adjusted by HCl and NaOH to pH 8.5. The injected concentration of each analyte is 2 · 10^{-4} M, except that mesityl oxide is present at 2 10^{-3} M. Mesityl oxide was used to measure the electroosmotic flow coefficient. Benzoic acid was

TABLE I

THE EFFECT OF TEMPERATURE ON pH OF 0.1 M THAM AND 0.025 M HYDROGENCARBONATE BUFFER SOLUTIONS

Temperature (‘C)	pH	
	THAM	HCO;
30	8.44	8.56
40	8.29	8.60
50	8.06	8.64
60	1.92	8.73
70	7.71	8.79
80	7.70	8.87

used as the other marker to estimate the change of electrophoretic mobility as a function of the change in viscosity. The sample solution was raised to 10 cm high for hydrodynamic injection from the anodic end of the capillary for 8 s.

RESULTS AND DISCUSSION

In order to demonstrate the effect of voltage-induced pH change within the capillary tube on the electrophoretic mobilities of analytes, two electrophoresis buffers which have different temperature coefficients are chosen. THAM with a large temperature coefficient and hydrogencarbonate with a small temperature coefficient are suitable buffers in our study. From Table I, which lists actual experi-

mental measurements in our laboratory, the pH of THAM buffer at different temperatures can be estimated from the following equations

$$pH_2 = pH_1 - 0.023 \ (T_2 - T_1) \quad 40\text{–}50°C \quad (9)$$

$$pH_2 = pH_1 - 0.007 \ (T_2 - T_1) \quad 70\text{–}80°C \quad (10)$$

$$pH_2 = pH_1 - 0.015 \ (T_2 - T_1) \quad \begin{array}{l} 30\text{–}40 \ \text{and} \\ 50\text{–}70°C \quad (11) \end{array}$$

On the other hand, pH of the hydrogencarbonate buffer increases as temperature increases. The pH of bicarbonate buffer at different temperatures can be calculated from eqns. 12 and 13

$$pH_2 = pH_1 + 0.004 \ (T_2 - T_1) \quad 30\text{–}50°C \quad (12)$$

$$pH_2 = pH_1 + 0.007 \ (T_2 - T_1) \quad 50\text{–}80°C \quad (13)$$

The decrease in the viscosity of the buffer is significant as can be seen from the dramatic change in electroosmotic flow coefficient in both systems as the voltage changes from 10 to 25 kV. From Tables II and III, the fractional changes in electroosmotic flow coefficient and the electrophoretic mobility between 10 and 25 kV based on the two markers, mesityl oxide (neutral) and benzoic acid (completely dissociated), are 2.1 and 2.2 for THAM and 2.0 and 2.0 for hydrogencarbonate buffers, respectively. The results show that our markers are well suited for predicting the change of m_{eo} and m_{ep} due to the change in viscosity of the buffer solution. Also this shows that the dissociation effect of these two markers can be neglected in the range of voltage-induced pH change in this work. Based on the m_{eo}

TABLE II

ELECTROOSMOTIC FLOW COEFFICIENT *(m,,)* AND ELECTROPHORETIC MOBILITIES *(m,,)* OF ANALYTES IN 0.1 M THAM BUFFER SOLUTION AT DIFFERENT VOLTAGES

Units are in 10^{-4} cm $V^{-1} s^{-1}$. 1 = Mesityl oxide; 2 = 4-chlorophenol; 3 = 3-chlorophenol; 4 = 2-chlorophenol; 5 = 3-nitrophenol; 6 = 2,4-dichlorophenol; 7 = 3-methyl-4-nitrophenol; 8 = 4-nitrophenol; 9 = 2-nitrophenol; 10 = benzoic acid.

Voltage (kV)	m_{eo}	m_{ep}								
	1	2	3	4	5	6	7	8	9	10
10	-4.5	0.4	0.8	1.7	1.8	2.2	2.5	2.8	2.8	2.9
15	-5.2	0.3	0.7	1.7	1.8	2.4	2.9	3.3	3.3	3.4
20	-6.7	0.3	0.7	1.7	2.0	2.8	3.6	4.1	4.1	4.4
25	-9.5	0.2	0.7	1.3	1.8	2.7	4.4	5.1	5.3	6.3

TABLE III

ELECTROOSMOTIC FLOW COEFFICIENT (m_{eo}) AND ELECTROPHORETIC MOBILITIES (m_{ep}) OF ANALYTES IN 0.025 A4 HYDROGENCARBONATE BUFFER SOLUTION AT DIFFERENT VOLTAGES

Units are in 10^{-4} cm $V^{-1}s^{-1}$. 1–10 represent the same analytes as those in Table II.

Voltage (kV)	m_{eo}	m_{ep}								
	1	2	3	4	5	6	7	8	9	10
10	− 5.0	0.8	1.3	2.2	2.3	2.5	2.1	3.0	3.0	3.0
15	− 6.0	1.0	1.6	2.6	2.7	2.9	3.2	3.5	3.5	3.5
20	-1.1	1.3	1.6	2.6	2.7	7.9	3.2	3.5	3.5	3.5
25	-9.9	1.8	3.1	4.5	4.8	5.0	5.5	6.0	6.0	6.0

values obtained (mesityl oxide), one can calculate the temperature of the liquid at the various operating voltages by using eqn. 7. This is shown in Table IV.

TABLE IV

THE CALCULATED TEMPERATURE AND pH OF 0.1 M THAM AND 0.025 M HYDROGENCARBONATE BUFFER SOLUTIONS AT DIFFERENT VOLTAGES

Voltage	THAM		HCO;	
	Temperature (°C)	pH	Temperature (°C)	pH
10	32	8.41	28	8.54
15	40	8.29	37	8.59
20	54	8.00	51	8.65
25	76	7.73	66	8.77

Since the electrophoretic mobility of an analyte depends on the fraction of its free ion, the degree of dissociation should be determined. It is a function of pH as expressed in eqn. 8. Hence, in order to illustrate the effect of voltage-induced pH change, it is important to know the degree of dissociation of the analytes at different voltages. Based on the results shown in Tables II and III, the ratio of the degree of dissociation of analytes at different voltages can be calculated from eqn. 6. The results are shown in Tables V and VI. From Table V, it is obvious that the pH of THAM buffer decreases when the voltage increases since the ratio decreases. The results of observed and calculated values agree each other, which means that the trend of pH change we estimated is correct. The ratios decrease in the THAM system while they increase a little in the hy-

TABLE V

COMPARISON OF THE OBSERVED AND CALCULATED RATIOS OF THE FRACTIONAL DISSOCIATION (α) OF ANALYTES IN 0.1 M THAM BUFFER SOLUTION AT DIFFERENT VOLTAGES

1 = α_{15}/α_{10}; 2 = α_{20}/α_{10}; 3 = α_{25}/α_{10}; the subscript refers to kV operating voltage.

Analytes	Observed			Calculated		
	1	2	3	1	2	3
2-Nitrophenol	1.01	0.97	0.87	1.01	0.95	0.86
4-Nitrophenol	1.01	0.97	0.84	1.01	0.95	0.85
3-Methyl-4-nitrophenol	0.99	0.95	0.81	1.01	0.95	0.85
2,4-Dichlorophenol	0.93	0.84	0.56	1.03	0.81	0.59
3-Nitrophenol	0.85	0.73	0.46	1.06	0.72	0.46
2-Chlorophenol	1.12	0.86	0.46	1.07	0.68	0.41
3-Chlorophenol	1.19	0.92	0.46	1.10	0.61	0.34
4-Chlorophenol	0.64	0.49	0.23	1.11	0.59	0.32

TABLE VI

COMPARISON OF THE OBSERVED AND CALCULATED RATIOS OF THE FRACTIONAL DISSOCIATION (a) OF ANA-LYTES IN 0.025 M HYDROGENCARBONATE BUFFER SOLUTION AT DIFFERENT VOLTAGES

$1 = \alpha_{15}/\alpha_{10}$; $2 = \alpha_{20}/\alpha_{10}$; $3 = \alpha_{25}/\alpha_{10}$; the subscript refers to kV operating voltage.

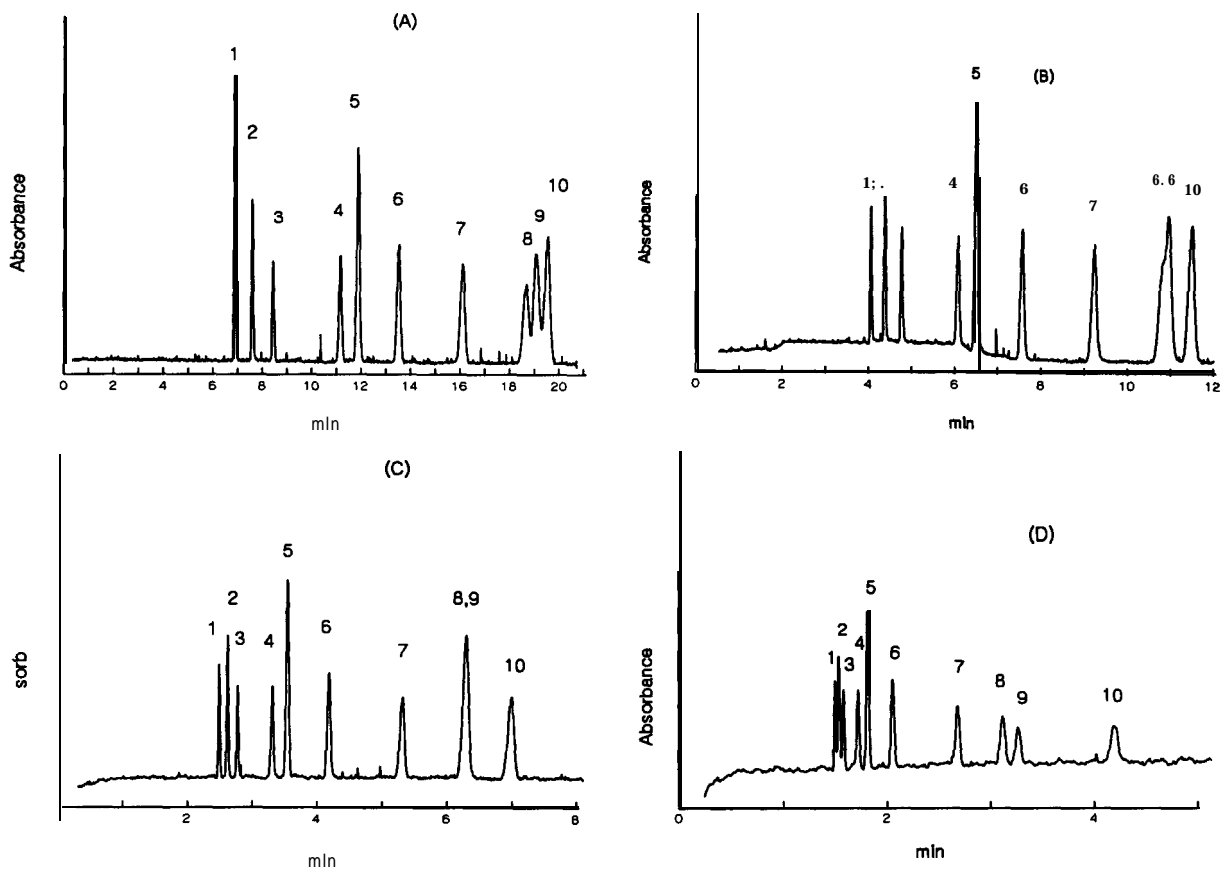| Analytes	Observed			Calculated		
	1	2	3	1	2	3
2-Nitrophenol	1.00	0.96	1.00	1.00	1.01	1.02
4-Nitrophenol	1.00	0.96	1.00	1.00	1.01	1.02
3-Methyl-4-nitrophenol	1.02	0.97	1.02	1.00	1.01	1.02
2,4-Dichlorophenol	0.99	0.94	1.00	1.02	1.04	1.08
3-Nitrophenol	1.01	0.97	1.02	1.04	1.09	1.17
2-Chlorophenol	1.01	0.96	1.02	1.05	1.12	1.24
3-Chlorophenol	1.05	1.03	1.19	1.09	1.20	1.45
4-Chlorophenol	1.07	1.04	1.13	1.11	1.24	1.56

Fig. 1. Separation of analytes in pH 8.5, 0.1 M THAM buffer solution at different voltages. Column: 130 μm I.D. × 360 μm O.D. × 55 cm total length (35 cm effective length). Detection wavelength = 218 nm. (A) 10 kV, (B) 15 kV, (C) 20 kV, (D) 25 kV. Peaks: 1 = mesityl oxide; 2 = 4-chlorophenol; 3 = 3-chlorophenol; 4 = 2-chlorophenol; 5 = 3-nitrophenol; 6 = 2,4-dichlorophenol; 7 = 3-methyl-4-nitrophenol; 8 = 4-nitrophenol; 9 = 2-nitrophenol; 10 = benzoic acid.

drogencarbonate system as the voltage increases. It is worth noting that the ratio changes significantly in the THAM buffer, implying that large voltage-induced pH changes can be obtained in the THAM buffer.

A group of phenolic compounds which have pK_a ranging from 7 to 9.5 are selected to demonstrate the separation improvement due to voltage-induced pH change. The effect of voltage-induced pH change on the separation of phenols can be shown in Figs. 1 and 2 for constant voltage operation. In the THAM system, 2-nitrophenol ($pK_a = 7.15$) and 4-nitrophenol ($pK_a = 7.17$) cannot be separated at the lower voltages while they can be separated at 25 kV. The result also agrees with our estimation of the pH of the buffer system in Table IV because the change in the degree of dissociation of an analyte is

more significant when the pH is near its pK_a. The other advantage is that electroosmotic flow increases as viscosity decreases, speeding up the separation. However, resolution among the first three analytes became worse at the high voltage.

In the hydrogencarbonate system, benzoic acid, 2-nitrophenol and 4-nitrophenol, which all have low pK_a values, cannot be completely separated. This is due to the insignificant increase in pH in the hydrogencarbonate buffer as voltage increases. Comparison of the result of separation ability in these two buffer systems again supports our conclusion that there is a higher voltage-induced pH change in the THAM buffer than in the bicarbonate buffer.

As discussed in the introduction, pH gradient is a well known method to improve the separation abil-
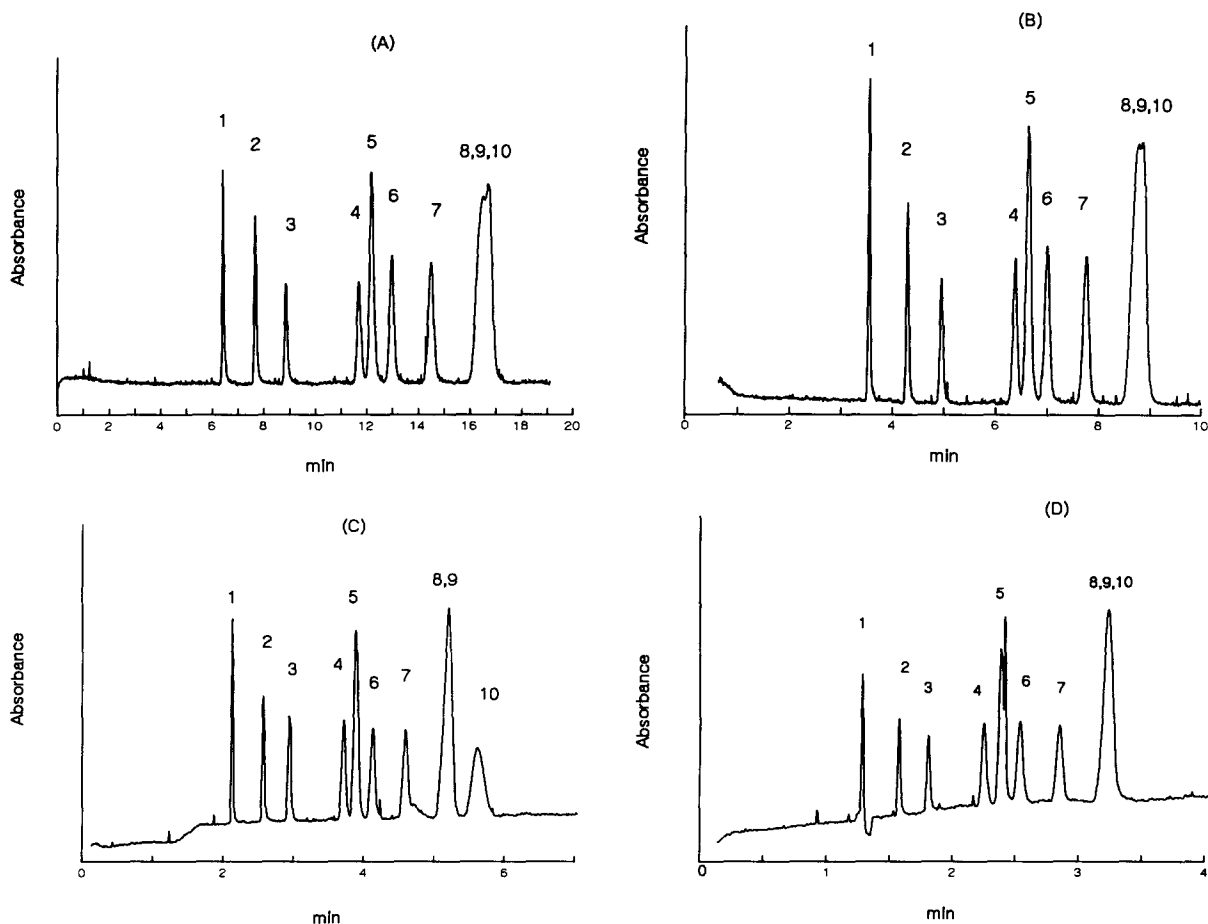

Fig. 2. Separation of analytes in pH 8.5, 0.025 M hydrogencarbonate buffer solution at different voltages. Other conditions as in Fig. 1.
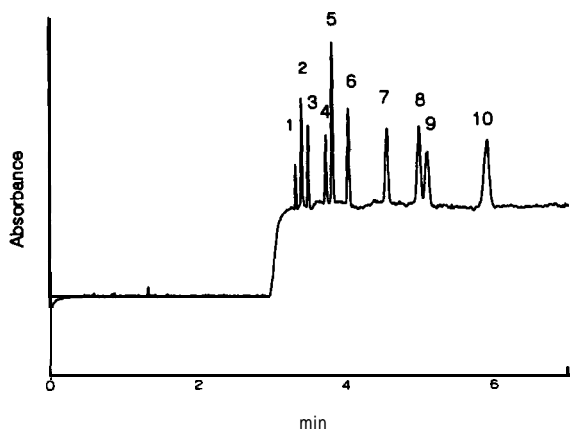
Fig. 3. The effect of voltage-induced pH program on the separation of phenols in pH 8.5, 0.1 *M* THAM buffer solution.

ity in CZE. To demonstrate the possibility of pH gradient generated via a step change in voltage, we use Fig. 3 as an example. Voltage programming starts from 15 kV for 3 min, then jumps to 25 kV for the remainder of the run. All analytes can be separated in 6 min with little overlap. There is a baseline shift due to the temperature change, as has been reported earlier [11]. From this result, we can conclude that voltage programming is advantageous for certain CE separations. As in the case of pH programming [9–11], groups of analytes that cannot be separated at any single pH can thus be resolved.

In summary, we have demonstrated the use of Joule heat to increase the temperature of the buffer during CZE separation. The resulting increase in temperature is able to generate a noticeable voltage-induced pH change and a change of the viscosity of 0.1 *M* THAM buffer to alter selectivity. Implementation is best in large-diameter capillaries, with high operating voltage, and at high buffer concentrations. In fact, efficient cooling of the capillary, e.g. in certain commercial CE instruments, is counterproductive in this mode of operation. There is the possibility that high Joule heating can reduce the

efficiency of the separation. However, for the systems studied here, degradation in efficiency was not observed. The use of voltage programming is in general simpler than the use of temperature programming [11]. The recycle time is also shorter since only the capillary and not the coolant needs to be returned to the initial temperature before the start of the next run.

ACKNOWLEDGEMENTS

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